AMENDMENTS

Amendments to the Specification

1. Please replace paragraph on page 1, lines 22-25 through page 2, lines 1-9 with the following paragraph:

   The above described behavior of voltage-gated Na^+ channels can be understood as follows. Na^+ channels reside in three major conformations or states. The resting or closed state predominates at negative membrane potentials (≤-60 mV). Upon depolarization, the channels enter the active state and open to allow current flow. The transition from resting to active states occurs within a millisecond after depolarization to positive membrane potentials. Finally during sustained depolarizations ([[<]] ≥1-2 ms), the channels enter a second closed or inactive state. Subsequent re-openings of the channels require a recycling of the channels from the inactive to the resting state, which occurs when the membrane potential returns to negative values. This means that membrane depolarization not only opens sodium channels but also causes them to close even during sustained depolarizations (Hodgkin and Huxley, 1952). Thus normal Na^+ channels open briefly during depolarization and are closed at rest (≤-60 mV).

2. Please replace paragraph on page 3, lines 1-21 with the following paragraph:

   Besides their importance under physiological conditions, Na^+ channels are also important under pathophysiological situations. For example they appear play a role in epileptic seizures, cardiac arrhythmias, and ischemia/hypoxia-induced cardiac and neuronal cell death (Taylor et al, 1997; Ragsdale et al, 1998). Importantly, the persistent Na^+ current appears to play a major role in generating the above mentioned cellular abnormalities (Stys, 1998; Taylor et al, 1997). For example persistent Na^+ current is ~~unregulated~~ upregulated in both cardiac and neuronal cells during hypoxia (Saint et al, 1996; Hammarstrom, 1998) and may ultimately lead to overload of cell Na^+ and calcium, conditions leading to cell death (Stys, 1998). Blockers of voltage-gated Na^+ channels have been shown to be effective in ameliorating cellular dysfunctions and death resulting from errant operation of voltage-gated sodium channels (Stys, 1998). However, in many cases these blockers inhibit both the normal (transient) and noninactivaing (persistent) Na^+ channels to the same extent. Significant block of normal transient Na^+ channels could seriously compromise cellular and organ function or may even cause death. Thus assuming that the persistent Na^+ current is the therapeutic target, it is important to develop drugs that will block this component of Na^+ current but not the normal transient current. However, in order to discern whether a compound selectively blocks the persistent over the transient Na^+ current conventional electrophysiological methods such as whole cell patch clamping or voltage clamping in oocyte preparations must be performed (Marty and Neher, 1995; Shih et al, 1998).

3. Please replace paragraph on page 4, lines 6-16 with the following paragraph:

   A method for identifying a Na^+ channel blocker in accordance with the present invention generally includes providing a cell containing a Na^+ channel ~~blocker~~. The channel ~~blocker~~ demonstrates both [[a]] transient and [[a]] persistent currents. The cell also includes a potassium ([[K]]K^+) channel and a Na/K ~~AtPase~~ATPase (Na^+ pump). A fluorescent dye is disposed into the well. The fluorescent dye is sensitive to change in

cell membrane potential in order to enable optical measurement of cell membrane potential. A Na^+ channel blocker, to be assayed, screened or otherwise identified, is added to the well and a stimulation current is passed through the cell in an amount sufficient to generate an action potential before and after the addition of the Na^+ channel blocker. Thereafter, a change in cell membrane potential is optically measured.

4. Please replace paragraph on page 5, lines 10-13 with the following paragraph:

   Figure 1 is a plot of current vs. time for voltage-gated Na^+ channels upon sustained depolarization ~~showing a combination of inactivating Na^+ channel~~ showing a combination of inactivating Na^+ channels transient current and non-inactivating Na^+ channel persistent current;

5. Please replace paragraph on page 6, lines 3-12 with the following paragraph:

   Figure 3 is a representation similar to Figure 2 of a cell enabling a ~~depolarization~~ hyperpolarization assay. The engineered cell contains K channels (denoted by gK for K conductance), Na^+ channels exhibiting normal transient (gNa) and noninactivating/persistent ($gNa_{persistent}$) currents and a Na^+ pump that maintains the cellular ion gradients. In this case gK = $gNa_{persistent}$ (equal conductance). Thus the membrane potential should be near midway between the equilibrium (Nernst) potential for [[K]] K^+ (E_K) and Na^+ (E_{Na}). Assuming cell and media [[K]] K^+ and Na^+ concentrations of 140 and 20 and 2 and 80 mM respectively E_K = -107mV and [[ENa^+]] E_{Na} = 35 mV. Thus the resting membrane potential will be near –36 mV. Upon blockage of persistent Na^+ channels E_m will hyperpolarize towards E_K (theoretically by as much as 70 mV);

6. Please replace paragraph on page 6, lines 14-23 with the following paragraph:

   Figure 4 is a representation of a cell similar to Figures 2 and 3 enabling a secondary depolarization following ouabain addition. The engineered cell contains [[K]] K^+ channels (denoted by gK for K conductance), Na^+ channels exhibiting normal transient (gNa) and noninactivating/persistent ($gNa_{persistent}$) currents and a Na^+ pump that maintains the cellular ion gradients. In this case gK >> $gNa_{persistent}$. Addition of ouabain will result in a small depolarization followed by a much larger secondary depolarization. In the absence of a significant Cl conductance or in Cl-free media Na^+ gained via persistent Na^+ channels can not be removed by the Na^+ pump therefore the cell gains Na^+ in exchange for [[K]] K^+. As the cell loses [[K]] K^+, E_K becomes more positive and the cell depolarizes. Blockers of persistent Na^+ channels will prevent the secondary depolarization; and

7. Please replace paragraph on page 8, lines 17-23 through page 9, lines 1-3 with the following paragraph:

   To begin the present assay the engineered cells are plated onto the wells 12 of the 96-386 well assay plate 10. The cells are in a Na-free physiological buffer that for example, can contain[[s]] in mM: 135 NMDG (N-methyl-d-glucamine)Cl, 5 KCl, 2.0 [[CaCl2]]$CaCl_2$, 1.5 mM [[MgCl2]]$MgCl_2$ and 20 mM Hepes pH adjusted to 7.4. The first addition to the wells will be a concentrated stock of KCl to elevate the [[K]] K^+ concentration enough to induce a small (10 mV or more) depolarization thus activating Na^+ channels. However, in the absence of extracellular Na^+ no additional depolarization will be seen. Within a

few milliseconds following [[K]] $\underline{K^+}$ addition the transient Na^+ channels will activate and then inactivate yet the channels generating the persistent Na^+ current will remain open.

8. Please replace paragraph on page 9, lines 19-23 through page 10, lines 1-12 with the following paragraph:

Figure 3 is a variation of the cell represented in Figure 2 to be used to screen blockers of persistent Na^+ channels. This assay takes advantage of the fact that persistent Na^+ channels are open at relatively negative membrane potentials as described previously (Stys, 1998). In this case the cell is engineered with [[K]] $\underline{K^+}$ and Na^+ channels such that the relative conductance of the [[K]] $\underline{K^+}$ channel and the portion of the Na^+ channels generating the persistent current are very similar. This will make the resting membrane potential lie approximately halfway between the equilibrium potential (Nernst) potential for Na^+ and that of [[K]] $\underline{K^+}$ (-40 to –20 mV). Under these conditions ~~blocking~~ noninactivaing Na^+ channels ~~(these~~ remain open[[)]] and depolarize the cell membrane. Thus, blocking the noninactivating Na^+ channels will hyperpolarize the membrane towards the equilibrium potential for [[K]] $\underline{K^+}$. Total block of persistent Na^+ channels could result in a significant hyperpolarization, as much as 50 to 60 mV (depending on the equilibrium potentials for Na^+ and [[K]] $\underline{K^+}$). In this case only one addition need be made and concerns about changes in cell volume due to changes in osmolarity (no concentrated stocks of KCl or NaCl will be added) are of no consequence since drug concentrations will be in the micromolar range. This screen should allow detection of agents that block persistent Na^+ current generated by noninactivating Na^+ channels.

9. Please replace paragraph on page 10, lines 13-17 with the following paragraph:

Figure 4 shows ~~the final~~ a variation of a cell for detecting blockers of persistent Na^+ channels in a high throughput screen. In this engineered cell there are [[K]] $\underline{K^+}$ channels, voltage gated Na^+ channels, containing a portion whose current is persistent, and a ouabain-sensitive Na/K ATPase (Na^+ pump). In this case ~~the~~ $gK >> gNa_{persistent}$. This means that the resting membrane potential will be near E_K.

10. Please replace paragraph on page 10, lines 18-23 through page 11, lines 1-7 with the following paragraph:

To start the assay, ouabain is added to the bath in order to block the Na^+ pump. This will lead to a small depolarization (due to blockage of the electrogenic Na^+ pump) and a large secondary depolarization. This secondary depolarization is the key to the assay and relies on the fact that the equilibrium potential for [[K]] $\underline{K^+}$ will become more positive. The rationale is as follows. Following ouabain addition, the cell will gain Na^+ via persistent Na^+ channels that are open at near resting membrane potential. In the absence of a Cl conductance (or in a [[Cl]] $\underline{Cl^-}$ free medium) the Na^+ gained by the cell will be electrically compensated for by an equimolar loss of [[K]] $\underline{K^+}$. Since the relative gK is large millimolar loss [[K]] $\underline{K^+}$ will result in a depolarization as its Nernst potential becomes more positive. The extent of the depolarization will depend on the amount of Na^+ gained and thus [[K]] $\underline{K^+}$ lost by the cell following the addition of ouabain. Compounds that block the persistent Na^+ channels will prevent this depolarization and do so in a dose-dependent manner.

11. Please replace paragraph on page 11, lines 14-22 through page 12, lines 1-2 with the following paragraph:

With reference to Figure 5 a well plate 10 includes wells 12 each containing a pair of silver/silver chloride or platinum electrodes 14, 16 in order to pass a stimulating current sufficient in magnitude to generate an action potential in the engineered cells discussed previously. The use of a fast voltage sensitive dye (FRET) as described above, enables an optical system 20 to measure membrane potential. Using this current passing method, (field stimulation), action potentials may be generated at will before and after the presence of a Na^+ channel blocker shown to inhibit persistent Na^+ channels. A dose response may then be performed to observe if the drug in question blocks the action potential and therefore a significant portion of the transient Na^+ current. In this way drugs that preferentially block persistent transient Na^+ channels may be discovered in a rapid high throughput format.

12. Please replace the Abstract on page 19, lines 3-11 with the following Abstract:

A method for identifying a Na^+ channel blocker, including providing a cell containing a Na^+ channel ~~blocker~~, demonstrating both a transient and a persistent current. The cell includes a potassium ([[K]] K^+) channel and a Na/K ~~AtPase~~ATPase (Na^+ pump). A fluorescent dye is disposed into the well. The fluorescent dye is sensitive to change in cell membrane potential in order to enable optical measurement of cell membrane potential. A Na^+ channel blocker, to be identified, is added to the well and a stimulating current is passed through the cell in an amount sufficient to generate an action potential before and after the addition of the Na^+ channel blocker. Thereafter, a change in cell membrane potential is optically measured.